PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C12N 07/00	A1	(11) International Publication Number: WO 85/04897 (43) International Publication Date: 7 November 1985 (07.11.85)

(21) International Application Number: PCT/US85/00720

(22) International Filing Date: 23 April 1985 (23.04.85)

(31) Priority Application Numbers: 602,946 643,729

(32) Priority Dates: 23 April 1984 (23.04.84) 24 August 1984 (24.08.84)

(33) Priority Country: US

(71) Applicant: UNITED STATES OF AMERICA, represented by THE UNITED STATES DEPARTMENT OF COMMERCE [US/US]; Washington, DC 20230 (US).

(72) Inventors: GALLO, Robert, C. ; 8513 Thornden Terrace, Bethesda, MD 20817 (US). POPOVIC, Mikulas ; 3 Pooks Hill Road #401, Bethesda, MD 20814 (US).

(74) Agent: OLIFF, James, A.; Parkhurst & Oliff, 277 South Washington Street, Alexandria, VA 22314 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: METHOD AND CELL LINE FOR CONTINUOUS PRODUCTION OF RETROVIRUSES (HTLV-III) RELATED TO AIDS

(57) Abstract

A cell system for the reproducible detection and isolation of human T-lymphotropic retroviruses (HTLV-family) with cytophatic effects (HTLV-III) from patients with the acquired immune deficiency syndrome (AIDS), pre-AIDS and healthy carriers. One neoplastic aneuploid T-cell line derived from an adult with lymphoid leukemia, termed HT, was susceptible to infection with HTLV-III, which is transformed and provides T-cell population which are highly susceptible t and permissive for HTLV-III, and convenient for large scale production, isolation, and biological detection of the virus. Other operational neoplastic T-cell lines which are positive for OKT4 marker, e.g., Molt 3, CEM, Ti7.4 and HUT78, can produce HTLV-III in a large amount and retain its unlimited capability for growth.

METHOD AND CELL LINE FOR CONTINUOUS PRODUCTION OF RETROVIRUSES (HTLV-III) RELATED TO AIDS

Technical Field

The present invention relates to a cell system for the reproducible detection and isolation of human T-lymphotropic retroviruses (HTLV-family) with cytopathic or cell killing effects (HTLV-III) from patients with the acquired immune deficiency syndrome (AIDS), pre-AIDS and in healthy carriers. One neoplastic aneuploid T-cell line, derived from an adult with lymphoid leukemia and termed HT, was susceptible to infection with HTLV-III, provided T-cell populations which are highly susceptible and permissive for HTLV-III, and is convenient for large scale production, isolation, and biological detection of the virus. Other OKT4 positive operational cell lines include Molt 3, CEM, Ti7.4 and HUT38, which after infection with HTLV-III virus can produce and preserve its capability for indefinite growth.

Background of the Invention

Epidemiologic data strongly suggest that acquired immune deficiency syndrome (AIDS) is caused by an infectious agent which is apparently horizontally transmitted by intimate contact or by blood products. Though the disease is manifested by opportunistic infections, predominantly Pneumocystis carcinii pneumonia and Kaposi's sarcoma, the underlying disorder affects the patient's cell-mediated immunity with absolute lymphopenia and reduced helper T-lymphocyte ($OKT4^+$) subpopulation(s). Moreover, before a complete clinical manifestation of the disease occurs, its prodrome, pre-AIDS, is frequently characterized by unexplained chronical lymphadenopathy and/or leukopenia involving a helper T-cell subset. This leads to the severe immune deficiency of the patient, suggesting that a specific subset of T-cells is the primary target for an infectious agent. Although patients with AIDS or pre-AIDS are often chronically infected with cytomegalovirus or hepatitis B

virus, for various reasons these appear to be opportunistic or coincidental infections apparently not linked to the immunological response deficiency.

It is believed that the cause of AIDS may be a virus from the family of human T-cell lymphotropic retroviruses (HTLV) which, prior to the present invention, comprised two major well characterized subgroups of human retroviruses, called human T-cell leukemia/lymphoma viruses, HTLV-I and HTLV-II. The most common isolate, HTLV-I, is mainly obtained from patients with mature T-cell malignancies. Seroepidemiological studies, in vitro biological effects, and nucleic acid hybridization data indicate that HTLV-I is etiologically associated with these malignancies, affecting adults primarily in the south of Japan, the Caribbean and Africa. HTLV of subgroup II (HTLV-II) was first isolated from a patient with a T-cell variant of hairy cell leukemia. To date, this is the only reported isolate of HTLV-II from a patient with a neoplastic disease. Virus isolation and seroepidemiological data show that HTLV of both subgroups can sometimes be found in patients with AIDS.

Evidence suggests that the retrovirus(es) of the HTLV family is an etiological agent of AIDS based on the following: (1) there is precedence for an animal retrovirus cause of immune deficiency (feline leukemia virus in cats); (2) retroviruses of the HTLV family are T-cell tropic; (3) they preferentially infect "helper" T-cells (OKT4^{+}); (4) they have cytopathic effects on various human and mammalian cells as demonstrated by their induction of cell syncytia formation; (5) they can alter some T-cell functions; (6) in some cases infection may result in selective T-cell killing; and (7) they are transmitted by intimate contact or through blood products. The presence of antibodies directed to cell membrane antigens of HTLV infected cells has been shown in sera of more than 40% of patients with AIDS (Essex et al., Science, 220:859 (1983)). This antigen has since

been defined as part of the envelope of HTLV (Schopbach, et al., Science, 224:503, May 4, 1984; and Lee, et al., Proc. Nat. Acad. Sci. USA, in press).

The original detection and isolation of the various HTLV isolates were made possible by two earlier developments: the discovery of T-cell growth factor (TCGF), also called Interleukin 2 (Il-2), which enabled the routine selective growth of different subsets of normal and neoplastic mature T-cells (Ruscetti, et al., J. Immunol., 119:131 (1977); and Poiesz, et al., Proc. Nat. Acad. Sci. USA, 77:6134 (1980)) and the development of sensitive assays for detection of retroviruses based on reverse transcriptase assays. The methods of HTLV isolation and transmission involved a cocultivation procedure using permissive T-cells for the virus. The use of normal human T-cells in cocultivation experiments preferentially yielded HTLV of both subgroups with immortalizing (transforming) capability for some of the target T-cells.

However, HTLV variants (now termed HTLV-III) lack immortalizing properties for normal T-cells, mainly exhibit cytopathic effects on the T-cells, and are now believed to be the cause of AIDS. In fact, such variants were frequently but only transiently detected using these normal T-cells as targets in cocultivation or cell-free transmission experiments. The cytopathic effect was overcome by finding a highly susceptible, permissive cell for cytopathic variants of HTLV, thus preserving the capacity for permanent growth after infection with the virus. The present invention discloses the identification and characterization of this new immortalized T-cell population and its use in the isolation and continuous high-level production of such viruses from patients with AIDS and pre-AIDS.

Early experiments identified one neoplastic aneuploid T-cell line, termed HT, derived from an adult with lymphoid leukemia, that was susceptible to infection

with the new cytopathic virus isolates.

This cell line is a sensitive target for transmission of these virus isolates (HTLV-III) and it allows continuous large-scale virus production and development of specific immunologic reagents and nucleic acid probes useful for comparison of these new isolates among themselves and with HTLV-I and HTLV-II. In addition to their differences in biological effects that distinguish them from HTLV-I and HTLV-II, HTLV-III also differs from these known HTLV subgroups in several immunological assays and in morphology. However, these new retroviruses are T4 lymphotropic and exhibit many properties similar to HTLV-I and HTLV-II, including similar properties of the reverse transcriptase, cross reactivity of structural proteins as determined by heterologous competition radioimmune assays with patients' sera and with animal hyperimmune sera, and induction of syncytia. These new retrovirus isolates are collectively designated HTLV-III. Together with detectable differences in some of their proteins and genetic information, HTLV-III's ability to kill T-cells clearly separates these variants from other members of the HTLV family.

Statement of Deposit

A cell line corresponding to the present invention, and denoted H9/HTLV-III_B, has been deposited in the American Type Culture Collection (ATCC, 12301 Parklawn Drive, Rockville, Maryland 20852-1776 USA) under ATCC No. CRL 8543 on April 19, 1984 This deposit assures permanence of the deposit and ready accessibility thereto by the public. H9 is a representative and preferred cell line in accordance with the invention. An additional deposit of Molt 3/-HTLV-III_B was made in the ATCC on August 15, 1984, under ATCC No. CRL 8602, and assures permanence of the deposit and ready accessibility thereto by the public.

General Description

A susceptible cell line HT was tested for HTLV

before *in vitro* infection and it was negative by all criteria, including lack of proviral sequences. Continuous production of HTLV-III is obtained after repeated exposure of parental HT cells (3 x 10^6 cells pretreated with polybrene) to concentrated culture fluids containing HTLV-III harvested from short term cultured T-cells (grown with TCGF) which originated from patients with pre-AIDS or AIDS. The concentrated fluids were first shown to contain particle associated reverse transcriptase (RT). When cell proliferation declined, usually 10 to 20 days after exposure to the culture fluids, the fresh (uninfected) HT parental cells are added to cultures. Culture fluids from the infected parental cell line were positive for particulate RT activity and about 20% of the infected cell population was positive in an indirect immune fluorescent assay (IFA) using serum from a hemophilia patient with pre-AIDS (patient E.T.). Serum from E.T. also contained antibodies to proteins of disrupted HTLV-III but did not react with proteins of HTLV-I or HTLV-II infected cells.

Specific Disclosure

As has been mentioned above, an aneuploid HT-cell line exhibited the desired prerequisites for the continuous propagation of HTLV-III. This cell line is a neoplastic aneuploid T-cell line derived from an adult patient with lymphoid leukemia, selected for its mature T-cell phenotype (OKT3$^+$ (62%), OKT4$^+$ (39%) and OKT8$^-$), as determined by cytofluorometry using a fluorescence-activated cell sorter. Cultures of these cells are routinely maintained in RPMI/1640 with 20% fetal calf serum and antibiotics. These cultures are shown in Example 1, Table 2. Clone H9 is preferred, with Clone H4 being secondarily preferred.

HTLV-III culture fluids are isolated from cultured cells of patients with acquired immune deficiency syndrome (AIDS). Peripheral blood leukocytes from these patients are banded in Ficoll-Hypaque, incubated in

growth media (RPMI 1640, 20% fetal bovin serum 0.29 mg/ml glutamine) containing 5 g/ml phytohemagglutinin (PHA-P) for 48 hours, at 37°C in a 5% CO_2 atmosphere. The leukocytes are then refed with growth medium containing 10% purified T-cell growth factor (TCGF); optionally, some of the cells also received rabbit antibody to alpha interferon. Cells and growth media from these lymphocytes are then assayed for the presence of HTLV subgroups I-III. Samples exhibiting more than one of the following were considered positive: repeated detection of a Mg^{++} dependent reverse transcriptase activity in supernatant fluids; virus observed by electron microscopy; intracellular expression of virus-related antigens detected with antibodies from seropositive donors or with hyperimmune serum; or transmission of particles, detected by reverse transcriptase assays or by electron microscopic observation, to fresh human core blood, bone marrow, or peripheral blood T-lymphocytes. All isolates not classified as either HTLV-I or HTLV-II by immunological or nucleic acid analysis were classified as HTLV-III. The cells in the HTLV-III producing cell cultures, characterized using established immunological procedures, are predominantly T-lymphocytes (E rosette receptor, OKT/3 and Leu/1 positive), with a T4 phenotype (OKT4, leu 3a positive). This process is also described by Gallo, et al., in Science, 220:865-867 (1983).

The infection of parental HT cells as well as other cloned cell populations occurs by exposure of these cells to concentrated or nonconcentrated culture fluids (cell-free infection) from T-cell cultures from AIDS or pre-AIDS patients, or by cocultivation; that is, HT cells are infected by exposure to HTLV-III positive T-cell cultures. The usual cell-free infection procedure is as follows: 2 to 5 x 10^6 cells are treated with polybrene (2 g/ml) or DEAE dextran for 30 minutes in a CO_2 incubator at 37°C, and then exposed to the virus inoculum (0.1 to 1 ml) for one hour in the incubator (CO_2/37°C).

The cells are kept in suspension by shaking at regular intervals. After one hour of incubation a regular gr wth medium is added. The positivity of infected cultures for HTLV-III is assessed after one, two, and three weeks of cultivation.

The infection of HT cells (clones) is also obtained by cocultivation procedures--HT cells are mixed in various proportions (usually 1:5) with short-term precultured T-cells (about 5 to 20 days) from AIDS or pre-AIDS patients. The positivity for HTLV-III was scored by the detection of viral antigens or viral nucleic acid sequences in the infected recipient cells at various intervals (7, 14, 21 days, etc.) after co-cultivation. The mixed cultures are maintained in growth medium for several months.

Co-culturing applies to a standard procedure for treating cells. Standard conditions for growing cells are 37°C in an atmosphere of 95% humidified air and 5% CO_2. In operation a donor cell from human blood yields a virus which acts on the target cells (HT neoplastic aneuploid T-cells) to yield a co-culture. The co-culture of retrovirus positive cells yields the virus and preserves permanent growth, i.e. growth in perpetuity (as HTLV-III and HT9); i.e., immortalized conditions.

In addition to H9, additional permissive cells have been found and identified which will permit co-culture of the viral HTLV-III positive cells; these are Molt 3 and HUT78 cells and also CEM and Ti7.4. The latter, Ti7.4, however, is a herpes-like virus and excludable from use on that basis. Characteristics of cells noted, both positive and negative effect, are listed in the following Table 1.

Table 1

HTLV-III Permissive Cells

Cells	Characteristics	IFA % for HTLV-III	RT Activity (rAdT/dAdT)
Molt 3	T (OKT4)	58	950000/ 5000
CEM	T "	53	84000/15000
Ti7.4	T "	22	900000/10000
HUT78	T "	64	150000/12000
CF-2	B	0	1500/ 5000
Dandie	B	0	6000/ 6000
Raji	B	0	8500/ 5500
K562	Erytr. leu.	0	55000/19000
HL60	Promyeloc. leu.	0	6000/ 5000

Example 1

As shown in Table 2 below, single cell HT clones were isolated as described by Popovic, et al., in Neoplasma, 18:257 (1971), and Bach, et al., Immunol. Rev., 54:5 (1981) from a long-term cultured aneuploid HT-cell line exhibiting mature T-cell phenotype ($OKT3^+$ (62%), $OKT4^+$ (39%) and $OKT8^-$) as determined by cytofluorometry using a fluorescence-activated cell sorter. The cultures were routinely maintained in RPMI/1640 with 20% fetal calf serum and antibiotics. The terminal cell density of the parental cell culture, seeded at a concentration of 2 x 10^5 cells/milliliter of culture media, was in the range of 1x10^6 to 1.5 x 10^6 cells/ml after 5 days of culture.

For detection of multinucleated cells, cell smears were prepared from cultures 6 and 14 days after infection and stained with Wright-Giemsa. Cells having more than 5 nuclei were considered as multi-nucleated cells. Cloned cells from uninfected cultures also contained some multi-nucleated giant cells as well; however,

ring formation was lacking and the number of these cells was much less (0.7% to 10%).

Immunofluorescence positive cells were washed with phosphate-buffered saline (PBS) and resuspended in the same buffer at a concentration of 10^6 cells per milliliter. Approximately 50 λ of cell suspension were spotted on slides, air dried, and fixed in acetone for 10 minutes at room temperature. Slides were stored at -20°C until use. Twenty microliters of either hyper-immune rabbit antiserum to HTLV-III (diluted 1/2000 in PBS) or serum from the patient (E.T.) diluted 1/8 in PBS was applied to cells and incubated for 50 min. at 37°C. The fluorescein-conjugated antiserum to rabbit or human immunoglobulin G was diluted and applied to the fixed cells for 30 minutes at room temperature. Slides then were washed extensively before microscopic examinations. The uninfected parental cell line as well as the clones were consistently negative in these assays.

To determine reverse transcriptase activity, virus particles were precipitated from cell-free supernatant as follows: 0.4 ml of 4M NaCl and 3.6 ml of 30% (wt/vol.) polyethylene glycol (Carbowax 6000) were added to 8 ml of harvested culture fluids and the suspension was placed on ice overnight. The suspension was centrifuged in a Sorvall RC-3 centrifuge at 2000 rpm at 4°C for 30 min. The precipitate was resuspended in 300 μl at 50% (vol/vol) glycerol (25 mM Tris-HCl, pH 7.5/5mM dithiothreitol/150 mM KCl/0.025% Triton X-100). Particles were disrupted by addition of 100 μl of 0.9% Triton X-100/1.5M KCl. Reverse transcriptase (RT) assays were performed as described by Poiesz, et al., Proc. Nat. Acad. Sci. USA, 77:7415 (1980) and expressed in cpm per milliliter culture medium.

Table 2

Response of Cloned T-Cell Populations to HTLV-III Infection

Characteristics	Clones							
	H3	H4	H6	H9	H17	H31	H35	H38
Total cell number (x 10^6)								
6 days after infection	1	1.5	1.5	0.3	0.4	0.3	0.5	1.8
14 days after infection	2.2	7.3	7.5	10.0	4.7	5.0	4.5	3.2
Multinucleated cells (%)								
6 days after infection	24	42	32	7	13	14	30	45
14 days after infection	45	48	45	30	22	45	60	60
Immunofluorescence positive cells (%)								
6 days after infection								
Rabbit antiserum to HTLV-III	55	56	32	32	39	21	10	87
Patient serum (E.T.)	56	29	21	ND	ND	ND	ND	73
14 days after infection								
Rabbit antiserum to HTLV-III	50	74	60	97	71	40	20	80
Patient serum	45	47	56	78	61	43	22	89
Reverse transcriptase activity (x 10^4 cpm/ml)								
6 days after infection	2.4	1.8	2.1	4.1	2.6	1.4	1.7	2.5
14 days after infection	16.2	18.1	16.1	20.2	17.1	13.4	15.1	18.2

ND = not done

Example 2

As shown in Table 3 below, cocultivation with H4 recipient T-cell clone was performed with fresh mononuclear cells from peripheral blood of patients RF and SN, respectively. In the case of patients BK and LS cocultivation was performed with T-cells grown in the presence of exogenous TCGF (10% V/V) for 10 days. The ratio recipient/donor (patients') cells was 1:5. The mixed cultures were maintained in RPMI/1640 (20% FCS and antibiotics) in the absence of exogenous TCGF. Cell-free infection of H9 T-cell clone was performed using concentrated culture fluids harvested from T-cell cultures of the patient WT. The T-cell cultures were grown in the presence of exogenous TCGF for two weeks before the culture fluids were harvested and concentrated. Cells of H9 clones were pretreated with polybrene (2 μg/ml) for 20 min. and 2 x 10^6 cells were exposed for one hour to 0.5 ml of 100-fold concentrated culture fluids positive for particulate RT activity.

HTLV-III virus expression in both cocultured and cell-free infected cell cultures were assayed approximately one month after *in vitro* cultivation. There was considerable fluctuation in HTLV-III expression (see Table 3). For details of both reverse transcriptase (RT) assays and indirect immunofluorescence assays (IFA) see Example 1.

Table 3

Isolation of HTLV-III from Patients with Pre-AIDS and AIDS

Patient	Diagnosis	Origin	Virus Expression: RT Activity (x 10^4 cpm)	Virus Expression: IFA with Rabbit Serum (% Positive	Virus Expression: IFA with Human Serum (ET) (% Positive)	EM
RF	AIDS (heterosexual)	Haiti	0.25	80	33	ND
SN	Hemophiliac (lymphadenopathy)	U.S.	6.3	10	ND	+
BK	AIDS (homosexual)	U.S.	0.24	44	5	+
LS	AIDS (homosexual)	U.S.	0.13	64	19	+
WT	Hemophiliac (Lymphadenopathy)	U.S	3.2	69	ND	ND

RT = reverse transcriptase
IFA = immunofluorescence assays
EM = electron microscopy
ND = not done

Example 3

To select for high permissiveness for HTLV-III and to preserve permanent growth and continuous production of virus, extensive cloning of the HT parental T-cell population was performed. A total of 51 single-cell clones was obtained by both capillary and limited dilution techniques using irradiated mononuclear cells from peripheral blood of a healthy donor as a feeder. The growth of these cell clones was compared after HTLV-III infection. A representative example of response to virus infection of 8 T-cell clones which are susceptible to and permissive for HTLV-III is shown in Table 2. In parallel experiments, 2 x 10^6 cells of each T-cell clone were exposed to 0.1 ml of concentrated virus. Then cell growth and morphology, expression of cellular viral antigen(s), and RT activity in culture fluids were assessed 6 and 14 days after infection. Although all 8 clones were susceptible to and permissive for the virus, there were considerable differences in their ability to proliferate after infection. The cell number decreased by 10% to 90% from the initial cell count within 6 days after infection, and a high proportion of multinucleated (giant) cells were consistently found in all 8 infected clones. The percentage of T-cells positive for viral antigen(s) determined by immunofluorescent assays with serum from AIDS patient (E.T.) and with hyperimmune rabbit serum raised against the whole disrupted HTLV-III ranged from 10% to over 80%. Fourteen days after infection, the total cell number and the proportion of HTLV-III positive cells increased in all 8 clones. The virus positive cultures consistently showed round giant cells which contained numerous nuclei. These multinucleated giant cells are similar to those induced by HTLV-I and HTLV-II except that the nuclei exhibit a characteristic ring formation. Electron microscopic examinations showed that the cells released considerable amounts of virus.

Example 4

To determine whether HTLV-III is continuously produced by the infected T-cells in long-term cultures, both virus production and cell viability of the infected clone, H4, were followed for several months. Although the virus production fluctuated, culture fluids harvested from the H4/HTLV-III cell cultures at approximately 14-day intervals consistently exhibited particulate RT activity which has been followed for over 5 months. The viability of the cells ranged from 65% to 85% and doubling time of the cell population, which is called H4/HTLV-III, was approximately 30-40 hours. Thus, this permanently growing T-cell population can continuously produce HTLV-III.

The yield of virus produced by H4/HTLV-III cells was assessed by purification of concentrated culture fluids through a sucrose density gradient and assays of particulate RT activity in each fraction collected from the gradient. The highest RT activity was found at density 1.16 g/ml, similar to other retroviruses.

Industrial Applicability

The preferred cell line which is a product of the present invention (H9/HTLV-III_B) is presently useful for the production of vaccines for the relief of AIDS and for the detection of antibodies to the virus in blood samples.

WHAT IS CLAIMED IS:

1. A method for producing HTLV-III virus, characterized in that a target T-cell is infected with HTLV-III virus, the normal cytopathic effect of HTLV-III is overcome and the immortal growth capacity of the target T-cell is preserved.

2. A method according to claim 1, characterized in that the target T-cell is a target OKT4$^+$ T-cell.

3. A method according to claim 1, characterized in that the target T-cell is an HT clone.

4. A method according to claim 3, characterized in that the HT clone is an H4 clone.

5. A method according to claim 3, characterized in that the HT clone is an H9 clone.

6. A method according to claim 1, characterized in that the HTLV-III virus comprises variants of human T-lymphotropic retrovirus which exhibit cytopathic effects and are non-transforming.

7. A method according to any one of claims 1-6, characterized in that the target T-cell is a neoplastic aneupioid T-cell.

8. A method according to claim 7, characterized in that the target T-cell is from a T-cell line derived from lymphoid leukemia.

9. A method according to claim 8, characterized in that the T-cell line has a mature T-cell phenotype of OKT3$^+$ (62%), OKT4$^+$ (39%) and OKT8$^-$.

10. A method according to claim 2, characterized in that the target T-cell is selected from Molt 3, CEM, Ti7.4 and HUT78 cells.

11. A method according to claim 8, characterized in that the cell line is capable of continuous large-scale production of HTLV-III.

12. A method according to any one of claims 1-6, 9 and 10 characterized in that the target T-cell is infected by cocultivation with the HTLV-III virus.

13. A method according to claim 7, character-

ized in that the target T-cell is infected by cocultivation with the HTLV-III virus.

14. A method according to claim 8, characterized in that the target T-cell is infected by cocultivation with the HTLV-III virus.

15. A method according to any one of claims 1-6, 9 and 10, characterized in that the target T-cell is infected with the HTLV-III virus by cell-free infection.

16. A method according to claim 7, characterized in that the target T-cell is infected with the HTLV-III virus by cell-free infection.

17. A method according to claim 8, characterized in that the target T-cell is infected with the HTLV-III virus by cell-free infection.

18. A method according to claim 11, characterized in that the target T-cell is infected with the HTLV-III virus by cell-free infection.

19. A cell line with a capacity for immortal growth, characterized in that the cell line contains T-cells infected with HTLV-III virus.

20. A T-cell line according to claim 19, characterized in that the T-cell line is H9/HTLV-III_B (ATCC Accession No. CRL 8543).

21. A T-cell line according to claim 19, characterized in that the T-cells are $OKT4^+$ permissive cells.

22. A T-cell line according to claim 21, characterized in that the $OKT4^+$ permissive cells comprise at least one of Molt 3, CEM, Ti7.4 and HUT78 cells.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/00720

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. Cl. 435/235
INT. Cl4 C12N 07/00

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
	435/235, 239, 240, 241, 948

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

BIOSIS, MEDLINE, EXCERPTA MEDICA, 1979-1984, HTLV III OR AIDS AND VIRUS, AND CULTURE OR CULTIVATE.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X,P	N, SCIENCE 224, 4 MAY 1984, POPOVIC ET AL, "DETECTION, ISOLATION AND CONTINUOUS PRODUCTION OF CYTOPATHIC RETROVIRUSES (HTLV-III) FROM PATIENTS WITH AIDS AND PRE-AIDS" PAGES 497-500	1-22
X,P	N, SCIENCE 224, 4 MAY 1984, SARNGADHARAN ET AL, "ANTIBODIES REACTIVE WITH HUMAN T-LYMPHOTROPIC RETROVIRUSES (HTLV-III) IN THE SERUM OF PATIENTS WITH AIDS" PAGES 506-8	1-22
X,P	N, SCIENCE 225, 24 AUGUST 1984, LEVY ET AL, "ISOLATION OF LYMPHOCYTOPATHIC RETROVIRUSES FROM SAN FRANCISCO PATIENTS WITH AIDS" PAGES 840-2	1-22
X	N, SCIENCE 220, 20 MAY 1983, GALLO ET AL, "ISOLATION OF HUMAN T-CELL LEUKEMIA VIRUS IN ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)" PAGES 865-7	1-22
A	N, SCIENCE 220, 20 MAY 1983, GELMANN ET AL, "PROVIRAL DNA OF A RETROVIRUS, HUMAN T-CELL LEUKEMIA VIRUS, IN TWO PATIENTS WITH AIDS" PAGES 862-5	1-22

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
3 JULY 1985	11 JUL 1985
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	John E. Tarcza JOHN E. TARCZA

Form PCT/ISA/210 (second sheet) (October 1981)

International Application No. PCT/US85/00720

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	N, SCIENCE 220, 20 MAY 1983, BARRE-SINOUSSI ET AL, "ISOLATION OF A T-LYMPHOTROPIC RETRO-VIRUS FROM A PATIENT AT RISK FOR ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)" PAGES 868 TO 71.	1-22
A	N, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA 80, NOVEMBER 1983, CHEN ET AL, "HUMAN T-CELL LEUKEMIA VIRUS TYPE II TRANSFORMS NORMAL HUMAN LYMPHOCYTES" PAGES 7006-9	1-22
A	N, SCIENCE 220, 20 MAY 1983, ESSEX ET AL, "ANTIBODIES TO CELL MEMBRANE ANTIGENS ASSOCIATED WITH HUMAN T-CELL LEUKEMIA VIRUS IN PATIENTS WITH AIDS" PAGES 859-62	1-22